

**Material & Methods** Ten histologic sections of human coronary plaque rupture were contrasted with ten cases of stable fibrocalcific plaques for the presence of peri-adventitial fat macrophages (Kp-1 positive cells). Quantitative morphometry was performed.

**Results.** The ten plaque rupture cases had a markedly greater macrophage infiltration as evidenced by number/mm<sup>2</sup> peri-adventitial fat. Moreover, the highest density in fibrocalcific cases (56/mm<sup>2</sup>) was lower than the lowest density in cases of plaque rupture (171/mm<sup>2</sup>). As expected, plaque rupture sections showed typical features of thin cap fibroatheroma with large lipid cores and high density of plaque macrophages that were accompanied by a significant macrophage infiltrate in the adventitia and peri-adventitial fat. S100 and Toluidine blue staining ruled out the possibility that these cells might represent dendritic cells or mast cells, respectively.

**Conclusion.** This study shows the presence of active macrophages in the peri-adventitial fat of human coronary arteries. These cells were present in much greater quantity in the setting of plaque rupture as opposed to burnt-out fibrocalcific plaques. There appeared to be no significant difference in macrophage density between adventitia and peri-adventitial fat. Peri-adventitial fat inflammation might play a significant pathogenic role in atherosclerosis progression and complications.

11:00 a.m.

#### 841-3 Apolipoprotein A1 Mimetic Peptide Reduces Accelerated Atherosclerosis in a Vein Graft Bypass Model in ApoE (-/-) Mice

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**BACKGROUND:** ApoA-1 mimetic peptide (DWFKAFYDKVAEKFEAF or D4F) reduces diet-induced aortic atherosclerosis. Whether D4F is effective in reducing accelerated atherosclerosis in vein graft is unknown. We studied the effect of intraperitoneal (IP) administration of D4F on accelerated atherosclerosis in a murine vein graft bypass model.

**METHODS:** Right carotid artery of hypercholesterolemic apoE (-/-) mice were grafted with a segment of IVC from donor mice at 16 weeks of age. Treatment group (n=10) received IP injection of 50 mcg D4F peptide daily for 4 weeks after surgery whereas control group (n=7) received saline injections. The grafts, heart and aorta were harvested and sectioned for morphometric and immunohistochemical analysis and plasma collected for cholesterol and D4F levels and lipoprotein fractionation analysis.

**RESULTS:** D4F treatment significantly reduced plaque size in the vein graft but not in the aortic sinus or aorta. D4F also reduced lipid content in the vein graft but not in the aortic sinus plaques (Table). There were no difference in cholesterol levels or lipoprotein fractionation or plaque phenotypes in the vein graft or aortic sinus plaques between the groups.

**CONCLUSION:** Four week treatment of Apo A-1 mimetic peptide reduces accelerated atherosclerosis in vein graft but has no effect on native spontaneous atherosclerosis in apoE (-/-) mice.

Group	Cholesterol (mg/dl)	vein graft plaque size (mm <sup>2</sup> )	lipid content in vein graft (%)	aortic sinus plaque size (mm <sup>2</sup> )	Lipid content in aortic sinus (%)	D-4F conc. (pmole/ml)
Control	1424±467	0.96±2.03	12.4±6.5	0.39±0.12	17.5±6.2	ND
D4F	1273±294	0.55±0.37*	6.4±3.8*	0.50±0.09	18.0±6.6	3767±1183

\*: p<0.05, by t-test

11:15 a.m.

#### 841-4 Bach1 Is a Key-Repressor of Heme Oxygenase-1 and Regulates Cell Proliferation

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**Background:** Heme oxygenase-1 (HO-1) protects cells from various insults including oxidative stress and its transcriptional induction by various stresses provides an important cellular adaptive defense mechanism. Recently we found that Bach1 is a physiological repressor of HO-1 (EMBO J., 2002). Though some investigators reported that HO-1 protects against vascular proliferation, the role for Bach1 in cell growth is poorly understood. The aim of this study is to elucidate the association between Bach1 and HO-1 expression and to examine the effect of bach1 ablation on cell proliferation.

**Methods:** We have developed *bach1* knockout (KO) mice and isolated aortic smooth muscle cells (SMC), macrophages, and embryonic fibroblasts (EF). Using RT-PCR method, western blotting and immunofluorescence staining, we analyzed the expression of HO-1 comparing them with that in wild type (WT) cells. Using retrovirus system we studied whether re-introduction of bach1 represses the expression HO-1 in KO cells or not. We also investigated effect of *bach1* ablation on cell proliferation using BrdU labeling assay both atmospheric levels (20%), which is oxidative condition for EF, and physiological levels of oxygen (3%). We detected the senescent cells with senescence  $\beta$ -galactosidase staining.

**Results:** Expression of HO-1 was increased in KO SMC, macrophages and EF as compared with WT cells (4.2-fold and p<0.01; 3.6-fold and p<0.05; 3.6-fold and p<0.01, respectively). While HO-1 expression was induced in WT cells when stimulated with Cd or hemin, no further induction was observed in the KO cells. Thus, HO-1 is fully activated in the absence of *bach1* function. Expression of HO-1 was clearly inhibited in KO cells upon *bach1* gene delivery. In 20% O<sub>2</sub>, proliferation of KO SMC and EF significantly decreased in both cell counting and in BrdU labeling assays. In 3% O<sub>2</sub>, KO EF prolifer-

ated as well as WT cells. When cultured in 20% O<sub>2</sub>, KO EF underwent senescence more frequently than WT cells.

**Conclusion:** Inactivation of *bach1* leads to increased expression of HO-1 and anti-proliferative effect. Bach1 is an important molecule for the response of oxidative stress. Bach1 may represent a novel molecular target in anti-atherosclerotic therapy.

11:30 a.m.

#### 841-5

#### Attenuation of Human Atherosclerotic Plaque Inflammation by PPAR- $\gamma$ Agonist and NF- $\kappa$ B Inhibitor

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**Background:** Although inflammation plays an important role in formation, progression and instability of atherosclerotic plaque, its inflammatory properties have not been fully examined. Since the reduction of plaque inflammation may improve clinical outcomes in high-risk individuals, we examined inflammatory properties of human atherosclerotic plaque, and evaluate anti-inflammatory potential of PPAR- $\gamma$  Agonist and NF- $\kappa$ B Inhibitor in an ex-vivo organ culture system utilizing advanced human atherosclerotic plaque for drug discovery.

**Methods:** Human atherosclerotic plaques (CaP; n=20) were obtained during carotid endarterectomy. Unaffected vessels (radial arteries and saphenous veins: NV; n=8) were harvested during elective CABG surgery. Tissue expression of inflammatory mediators was analyzed by real-time RT-PCR (mRNA), and ELISA at baseline, after 2 and 24 hours incubation in the absence or presence of PPAR- $\gamma$  agonist (rosiglitazone, 20  $\mu$ M, n=14) or NK- $\kappa$ B inhibitor (MG-132, 100  $\mu$ M, n=9).

**Results:** At the baseline, CaP exhibited higher expression of IL-1 $\beta$  (7.8±2.1 vs. 1.9±0.4; p<0.01), TNF- $\alpha$  (0.9±0.1 vs. 0.3±0.1; p<0.01) and MCP-1 (0.9±0.1 vs. 0.3±0.1; p<0.01) on mRNA level, as compared to NV (respectively). After stimulation, rosiglitazone reduced TNF- $\alpha$  expression (4.4±0.9 vs. 2.3±0.4; p<0.05), whereas NF $\kappa$ B inhibitor attenuated TNF $\alpha$  (4.6±0.9 vs. 0.7±0.2; p<0.05), IL-6 (91.2±38 vs. 2.5±0.4; p<0.05), IL-8 (17.1±1.3 vs. 1.7±0.7; p<0.001), and MCP-1 (54.0±13.4 vs. 3.0±0.5; p<0.05) expression on protein and mRNA levels (not shown).

**Conclusions:** 1. Atherosclerotic plaque releases higher levels of inflammatory mediators, as compared to normal vessel; 2. Inflammatory properties of atherosclerotic plaque are modifiable, that provides experimental platform for novel drug discovery in atherosclerosis; 3. Further evaluation of therapy targeting specific inflammatory pathways is necessary.

11:45 a.m.

#### 841-6

#### Interleukin-18 and Interleukin-18 Binding Protein in Patients With Acute Coronary Syndromes

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**Background:** Interleukin-18 (IL-18) is a pro-inflammatory cytokine produced by macrophages that induces interferon- $\gamma$  production in T-cells, and acts with IL-12 to upregulate T-helper cell activity. In animal models IL-18 is a potent promoter of both atherogenesis and plaque instability, and among patients with coronary disease serum IL-18 is a predictor of subsequent cardiovascular death. IL-18 binding protein (IL-18BP) is a recently identified circulating high-affinity antagonist that binds to and neutralizes IL-18. We sought determine (1) The strength of the relationship between serum IL-18 concentration and clinical coronary events, and (2) the nature of the relationship between IL-18 and IL-18BP among patients with coronary artery disease.

**Methods:** Serum concentrations of IL-18, IL-18BP and other inflammatory mediators (IL-5, IL-8, IL-12, IL-13, anti-HSP60, GM-CSF, TNF, MCP-1, IFN- $\gamma$ ) were measured immediately prior to coronary angioplasty in 79 patients. Patients were grouped as acute coronary syndrome positive (ACS+) or negative (ACS-) based on presence or absence of elevated troponin I levels.

**Results:** No significant differences existed between ACS+ and ACS- patients with respect to clinical variables (including age, gender, prior MI, smoking, cholesterol, hypertension, diabetes). The IL-18 concentration was significantly greater among ACS+ vs. ACS- patients (452 ± 384 vs. 301 ± 172 pg/ml, p=0.017). There was, however, no significant difference between the groups with respect to IL-18BP levels, as reflected by an elevated ratio of IL-18 / IL-18BP among the ACS+ compared to ACS- patients (150 ± 145 vs. 89 ± 67, p=0.011). Of all mediators examined, only IL-18 was significantly associated with the presence of recent ACS.

**Conclusions:** IL-18 emerged as the most potent correlate of recent plaque instability among the mediators and clinical variables examined. The elevated serum IL-18 concentrations present in the ACS+ patients were not "opposed" by a concomitant rise in IL-18BP levels. These findings support a clinical association between IL-18 and plaque instability, and support further study of IL-18BP as a potential means to modulate the pro-inflammatory effects of IL-18.